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Insulin and IGF-1 Induce Different Patterns of Gene Expression in Mouse Fibroblast NIH-3T3 Cells: Identification by cDNA Microarray Analysis

JOELLE DUPONT, JAVED KHAN, BAO-HE QU, PAUL METZLER, LEE HELMAN, AND DEREK LEROITH

Section on Cellular and Molecular Physiology (J.D., B.-H.Q., D.L.), Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases; Cancer Genetics Branch (J.K., P.M.), National Human Genome Research Institute, National Cancer Institute, Molecular Regulation; and Molecular Oncology Section (L.H.), Pediatric Oncology Branch, NIH, Bethesda, Maryland 20892-1758

The IGF-1 receptor and the related insulin receptor are similar in structure and activate many of the same postreceptor signaling pathways, yet they mediate distinct biological functions. It is still not understood how the specificity of insulin *vs.* IGF-1 signaling is controlled. In this study, we have used cDNA microarrays to monitor the gene expression patterns that are regulated by insulin and IGF-1. Mouse fibroblast NIH-3T3 cells expressing either the wild-type human IGF receptor or the insulin receptor were stimulated with either IGF-1 or insulin, respectively. Thirty genes, 27 of which were not previously known to be IGF-1 responsive, were up-regulated by IGF-1 but not by insulin. Nine genes, none of which was previously known to be insulin responsive, were up-regulated by insulin but not by IGF-1. The IGF- and insulin-induced regulation of 10 of these genes was confirmed by Northern blot analysis. Interestingly, more than half of the genes up-regulated by IGF-1 are associated with mitogenesis and differentiation, whereas none of the genes specifically up-regulated by insulin are associated with these processes. Our results indicate that under the conditions used in this study, IGF-1 is a more potent activator of the mitogenic pathway than insulin in mouse fibroblast NIH-3T3 cells. (*Endocrinology* 142: 4969–4975, 2001)

THE POLYPEPTIDE HORMONES insulin and IGF-1 are closely related factors that are essential for normal metabolism and growth regulation. These peptides mediate their biological effects by binding to their respective transmembrane receptors on the surface of target cells. Insulin and IGF-1 are capable of cross-reacting with the insulin and IGF-1 receptors (IR and IGF-1R, respectively), but each receptor binds its own ligand with a 100- to 1000-fold higher affinity than that of the heterologous peptide. In addition, IGF-1, but not insulin, binds to specific IGF-binding proteins that also regulate IGF-1 activity (1). Although the IR and IGF-1R have certain shared functions, both *in vivo* and *in vitro* studies suggest that each receptor also has distinct biological roles (2–5). For example, IGF-1, acting through its cognate receptor, is not able to stimulate lipogenesis or to rescue the lethal phenotype in mice that lack the IR (6, 7). Thus, although IGF-1Rs can mediate some metabolic actions of IGF-1, the IGF-1R cannot fully compensate for the absence of IRs. Also, IGF-1R-deficient mice exhibit severe abnormalities in growth and differentiation and die at or immediately after birth (8). This indicates that the IR cannot functionally substitute for the lack of the IGF-1R. In addition, the IGF-1R can mediate cellular transformation when expressed in cells derived from IGF-1R-deficient mouse embryos, but the IR cannot (9).

Despite these divergent biological functions, the cell surface IR and IGF-1R share a high degree of identity in their primary and tertiary structures. Both receptors are composed of two extracellular α-subunits that include the ligand-binding domain and two transmembrane β-subunits that possess intrinsic tyrosine kinase activity (10, 11). The highest degree of homology between the two receptors is found in the tyrosine kinase domain (about 84%), whereas the region of greatest divergence between the IR and IGF-1R is found in the C-terminal domains, which share about 44% identity (12). The IR and IGF-1R are activated in a similar manner. Binding of the ligand to the α-subunits activates the IR or IGF-1R, leading to autophosphorylation of tyrosine residues within the β-subunits and subsequent enzymatic activation of the tyrosine kinase (10). All conserved tyrosine residues that are phosphorylated in the IR in response to insulin are also phosphorylated in the IGF-1R in response to IGF-1 (13, 14). In addition to the similarity in receptor structure, the IR and IGF-1R activate a highly similar set of downstream intracellular events. Both receptors phosphorylate various substrates on the same set of tyrosine residues, including IRS-1 (15, 16), IRS-2 (17, 18), IRS-3 (19, 20), IRS-4 (21), Gab-1 (22, 23), and Shc (24, 25). Consequently, the IR and IGF-1R activate many of the same signaling molecules, including those of the Ras-Raf-MAPK pathway (26, 27) and the PI3K pathway (28–30).

Thus, although both the IR and IGF-1R target many of the same intracellular substrates and activate similar signaling pathways, they are able to trigger distinct cellular responses. Therefore, it is important to ask how the specificity of insulin *vs.* IGF-1 signaling is achieved. In this study, we used cDNA microarrays to simultaneously monitor the expression levels of many genes to identify genes differentially regulated by insulin and IGF-1. NIH-3T3 mouse fibroblasts overexpressing either the wild-type human insulin or IGF-1 receptors were stimulated with either insulin or IGF-1, respectively.

Abbreviations: EST, Expressed sequence tag; IGF-1R, IGF-1 receptor; IR, insulin receptor.

We have identified a total of 39 genes that were specifically responsive to either IGF-1 or insulin. Most of these genes were not previously known to be regulated by either insulin or IGF-1. Analysis of these expression profiles revealed that IGF-1 primarily induced genes involved in mitogenesis or differentiation. In contrast, insulin specifically induced a broader spectrum of genes that, as a group, did not fall into any particular category. This study represents the first time that cDNA microarray technology has been used to define the specificity of insulin *vs.* IGF-1 signaling.

Materials and Methods

Cell culture

Two mouse fibroblast clones used in this study, NWTb3 and NWTc43, were developed in our laboratory as previously described (31, 32). These NIH-3T3 cell lines express the normal human IGF-1R at a level of about 4×10^5 receptors/cell (31, 32). IR cells are NIH-3T3 cells expressing the human wild-type IR at a level of about 2×10^6 receptors/cell (33). The IR cell line was a gift from Dr. S. Taylor (NIH, Bethesda, MD). NWTb3, NWTc43, and IR cells were derived in the same parental mouse embryonic fibroblast NIH-3T3 cell line that expresses about 16×10^3 IGF-1R/cell (31) and 5×10^3 IR/cell (33). All NIH-3T3 clones were routinely cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 300 μg/ml L-glutamine, and 500 mg/ml G418 (Geneticin, Life Technologies, Inc., Rockville, MD) in a humidified atmosphere of 95% air-5% CO_2 at 37 C. Cells were grown in 100-mm dishes, and once cells reached 75–80% confluence, dishes were rinsed twice with PBS and switched to serum-free medium containing 0.1% BSA, 20 mM HEPES, pH 7.5. Cells were serum starved overnight and were then stimulated with either 50 nM IGF-1 (for NWTb3cells) or 50 nM insulin (for IR cells) for 90 min at 37 C. The 90-min time period was chosen to minimize the chance of studying immediate early response genes or secondary events. After stimulation, cells were harvested and total RNA was extracted from cells using the TRIzol reagent (Life Technologies, Inc.), as described below.

cDNA microarrays

The mouse array is composed of 3899 detector elements. Of these, 315 are unclustered expressed sequences tags (ESTs), 630 are clustered ESTs, and 3004 are clustered, named genes. There is significant redundancy in the named gene portion of the set, with 2221 unique clusters represented. The clones were obtained from Research Genetics, Inc. (Huntsville, AL). PCR products from these clones were prepared and printed onto glass slides according to previously described protocols (34, 35).

RNA preparation, labeling, hybridization, and scanning

Total RNA was prepared from NWTb3 and IR cells by subjecting them to two extractions with TRIzol (Life Technologies, Inc.) according to manufacturer's recommended conditions. Total RNA was dissolved in 500 μl of water and concentrated to 17 μl using Microcon 30 (Amicon, Inc., Beverly, MA) before fluorescence labeling. Total RNA (100–200 μg) was converted to fluorescently labeled cDNA with either Cy-3 or Cy-5 (Amersham Pharmacia Biotech, Piscataway, NJ) and SuperScript II reverse transcriptase (Life Technologies, Inc.) exactly as described previously (34, 35). Imaging and image analysis were performed exactly as previously described (34, 35). Differentially expressed genes were defined as outliers if the calibrated red to green ratio was greater than 2.0 for all genes that had a minimal intensity of 2000 in either channel. The cutoff value of 2-fold is conventionally used by other investigators (36).

DNA sequencing and sequence analysis

The identities of differentially expressed genes in response to IGF-1 and insulin obtained after array hybridization were verified by DNA sequencing using vector-specific primers (either M13 forward or reverse primers). Cycle sequencing reactions with *Taq* DNA polymerase were performed with fluorescently labeled dideoxynucleotides (Dye-terminator, PE Applied Biosystems, Foster City, CA). Sequence database searches were performed with BLAST sequence comparison programs at the National Center for Biotechnology Information (http:/www.ncbi.nlm.nih.gov/BLAST). PCR products were used as a probe for the Northern blot analysis.

Northern blot analysis

Cells overexpressing the IGF-1R or IR were incubated in either the absence or presence of IGF-1 (NWTb3 or NWTc43 cells) or insulin (IR cells). Total RNA was isolated from these cells using the TRIzol reagent (Life Technologies, Inc.) as described above. Twenty micrograms of total RNA was separated by denaturing formaldehyde electrophoresis and then transferred overnight by capillary blot to positively charged nylon membranes. RNA was immobilized to membranes by UV cross-linking. Blots were prehybridized for 2 h at 42 C in a buffer containing 50% formamide, 5× Denhardt's solution, 1% SDS, 5× sodium saline citrate, and 100 μl/ml salmon sperm. Blots were then hybridized overnight at 42 C with 2×10^6 cpm/ml [^{32}P]dCTP-labeled DNA probe in a buffer containing 50% formamide, 2.5× Denhardt's solution, 1% SDS, 5× sodium saline citrate, 10× dextran sulfate, and 100 μl/ml salmon sperm. The probes were generated from DNA by PCR from sequence-verified IMAGE Consortium clones (Research Genetics, Inc.) and ^{32}P-labeled using the Rediprime labeling kit (Amersham Pharmacia Biotech). Finally, blots were washed under conditions of high stringency, and the ^{32}P-labeled probe that was hybridized was quantified using a PhosphorImager apparatus (FujiFilm, Stamford, CT). Autoradiography was also carried out at −70 C. The integrity and the quantification of different transcripts were assessed using the human RNA 18S probe from Ambion, Inc. (Austin, TX).

Results and Discussion

Despite the high degree of similarity in structure and substrate specificity, the IR and the IGF-1R do not appear to have redundant functions *in vivo*. However, the biochemical and physiological comparison of the two receptors is complicated by the fact that each ligand can cross-react with the other receptor and the fact that heterodimeric receptors can form when both receptors are expressed in the same cells. To overcome these problems, we have compared the effects of insulin and IGF-1 in NIH-3T3 fibroblasts overexpressing either human IR or IGF-1R. Cells overexpressing the IGF-1R (NWTb3 cells) or the IR (IR cells) were incubated in the presence or absence of IGF-1 or insulin, respectively. RNA was extracted and prepared for hybridization with the cDNA microarray as described in *Materials and Methods*. The color images of the hybridization results were generated by representing the Cy-3 fluorescent image as green and the Cy-5 fluorescent image as red and then merging the two color images. To ensure reproducibility of the microarray results, we repeated each experiment twice using different total RNA samples. The spots with signal intensities that were at least 2-fold different from control levels in both experiments were designated as genes that are differentially expressed in response to IGF-1 or insulin. Fig. 1 represents a typical hybridization result in which the cDNA probe derived from unstimulated NWTb3 cells was labeled with Cy-3 fluorochrome (*green*) and the cDNA probe from IGF-1-stimulated NWTb3 cells was labeled with Cy-5 fluorochrome (*red*). Spots with fluorescent signals that are strongly red (*e.g.* TDAG and Daxx, as shown in Fig. 1) indicate that expression of these genes is increased in response to IGF-1. Identical microarray plates were hybridized with similar fluorescently labeled cDNA probes derived from RNA from control (serum-deprived) and insulin-stimulated IR cells. The signal intensity ratios obtained for insulin *vs.* control in IR cells were compared with those obtained for IGF-1 *vs.* control in NWTb3 cells.

FIG. 1. Representative portion of cDNA microarray showing the effect of IGF-1 on gene expression patterns in NWTb3 cells. RNA from serum-starved NWTb3 cells was used to prepare cDNA labeled with Cy3-deoxyuridine triphosphate, and RNA from treated IGF-1 NWTb3 cells was used to prepared cDNA labeled with Cy5-deoxyuridine triphosphate. The control sample (serum-starved cells) corresponds to the *green* fluorochrome, and the experimental sample (cells treated with 50 nM IGF-1) corresponds to the *red* fluorochrome. These probes were mixed and cohybridized to the microarray as described in *Materials and Methods*. In this representative example, mRNAs that were up-regulated in response IGF-1 in NWTb3 cells are visualized as red spots. Two genes up-regulated by IGF-1, the TDAG51 and Daxx genes, are indicated.

Of the 2221 genes on the cDNA microarrays, we found that 30 genes were significantly induced by IGF-1 but not by insulin (Table 1), whereas only 9 genes and 1 EST were specifically up-regulated by insulin but not by IGF-1 (Table 2). Surprisingly, 27 of the 30 genes induced by IGF-1 had not been previously reported as IGF-1-responsive genes. Similarly, none of the genes induced by insulin were previously identified as insulin-responsive genes. We used Northern blots to confirm the changes in mRNA levels of 10 genes identified in the cDNA microarray analysis (Fig. 2). For Northern blots, probes derived from PCR products were amplified from plasmid DNAs containing the appropriate cDNAs. For all Northern blot experiments, RNA was isolated from separate sets of cells that were treated with IGF-1 or insulin (*i.e.* these experiments were independent of those used for the microarray technology). Moreover, we studied a second clone, NWTc43, that expresses similar levels of the wild-type human IGF-1R as NWTb3 (31). Our Northern blot results confirmed that all selected genes were regulated by IGF-1 and insulin, as predicted by the microarray analysis (Fig. 2). These results demonstrate that the cDNA microarray experiments accurately identified changes in gene expression mediated by IGF-1 and insulin. These findings are consistent with previous studies indicating that cDNA microarrays can predict changes in gene expression observed by Northern blot with high reliability (35–37). The microarray results also indicated that 13 genes were up-regulated and 3 genes were down-regulated by insulin and IGF-1, respectively (Tables 3 and 4). All of the genes that were identified as up-regulated by both insulin and IGF-1 in the cDNA microarray analysis appear to be more strongly stimulated by IGF-1 than by insulin (Table 3). This is not surprising, because it has been well established that IGF-1 is a more potent mitogen than insulin. It is important to note that the ratios obtained for some genes are quite close to the 2-fold cutoff value (*e.g.* MAK16, DBPA, and EDR in Table 1). Consequently, until confirmed by Northern blot analysis, these results must be interpreted with caution. The genes that were down-regulated by both hormones were similarly regulated by both IGF-1 and insulin (Table 4). These genes were not studied further because we were specifically interested in genes that were differentially regulated by IGF-1 and insulin. Our results are especially pertinent in view of the recent study by Fambrough and co-workers (38). In that study, the same set of 66 immediate early genes was found to be induced in fibroblasts by both the platelet-derived growth factor-β receptor and the fibroblast growth factor receptor, and a subset of these genes was induced by the epidermal growth factor receptor. These investigators concluded that an overlapping group of immediate early genes are induced by related growth factors that nevertheless have different biological actions. In contrast, in the present study, we found a number of genes to be differentially regulated by IGF-1 and insulin in fibroblasts.

The genes that were identified as regulated by IGF-1 and insulin are involved in various cellular functions, including cell proliferation, differentiation, and apoptosis, all of which are consistent with the known functions of these growth factors (Tables 1 and 2). Despite the known metabolic functions of these growth factors, insulin and IGF-1 significantly regulated only two genes that are associated with cellular metabolism. The cell type used in these studies could be related to this result. Fibroblasts are proliferative cells that may not have a well established cellular machinery to mediate metabolic functions, at least compared with other insulin-responsive cell types, such as adipocytes, myocytes, or hepatocytes. Interestingly, 18 of 30 genes up-regulated by IGF-1 in this study were previously reported to be involved in mitogenesis and differentiation in other contexts (Table 1). Only three of these genes, the Jun oncogene (39, 40), α-5 integrin (41), and early growth response-1 (42) have been previously reported to be responsive to IGF-1. For example, it is known that IGF-1 increases the level of α-5 integrin protein in lens epithelial cells, whereas insulin down-regulates α-5 integrin in normal human fibroblasts (41). In accordance with these findings, we found that IGF-1 but not

TABLE 1. Genes up-regulated specifically by IGF-1

	Symbol	Clone number	IGF-1	Insulin
Mitogenesis and differentiation				
IL-3 receptor, α-chain	IL-3Rα	445664	5.32	1.23
Colony-stimulating factor, macrophage	mCSF	634838	4.12	1.32
Glial cell line-derived neurotrophic factor	GNDF	425671	3.96	0.80
Integrin α-5 (fibronectin receptor)	Iα5	476908	3.55	0.94
Early growth response-1	EGR-1	608153	3.65	0.58
Jun oncogene	JUN	949554	3.01	1.11
Twist gene	TWIST	479367	2.95	1.54
Forkhead homolog 14	FKH-14	541099	2.91	1.08
Wee 1-like protein kinase	Wee-1	539548	2.75	1.95
IGF binding protein 10	IGF-BP10	557055	2.41	1.48
SRY box-containing gene 2	SRY-2	351033	2.39	0.59
IL-4 receptor α	IL-4Rα	721594	2.30	0.80
DNA-binding protein A	DBPA	602275	2.29	1.65
MAK16	MAK16	537328	2.27	1.70
Nerve growth factor-induced clone A-binding protein 2	NGFI-A BP-2	476298	2.31	1.25
Mothers against decapentaplegic-5	MAD5	551401	2.24	1.49
Early development regulator	EDR	616348	2.22	1.67
Ets variant gene 6 (TEL oncogene)	TEL	402134	2.21	0.97
Apoptosis				
T cell death-associated gene 51	TDAG51	694076	9.00	1.52
Fas-binding protein (Daxx)	Daxx	736796	5.99	1.55
Cellular processes				
Variant histone H3.3	vH3.3	618380	3.30	1.39
Kinesin heavy chain member 1A	Kin1A	492514	2.83	0.67
Chromatin nonhistone high mobility group protein	HGM-I(Y)	616054	2.64	1.15
Eukaryote release factor 1	eRF-1	572924	2.34	1.19
DEAD (aspartate-glutamate-alanine-aspartate) box polypeptide 5	DEAD5	537478	2.22	1.24
Splicing factor, arginine/serine 3	SRp20	595904	2.41	1.69
Metabolism				
Gibbon ape leukemia virus receptor-1	GLVR-1	335579	4.88	1.20
Glycerol phosphate dehydrogenase 1, mitochondrial	GPDH	351221	2.74	0.91
Others				
Nuclear factor erythroid derived 2, like 2	NF-E2	635541	2.90	0.86
Immediate early protein Gly96	Gly96	579574	2.46	1.13

TABLE 2. Genes up-regulated specifically by insulin

	Symbol	Clone number	IGF-1	Insulin
Morphogenesis and development				
α-B crystallin	CRYαB	605970	1.56	2.28
Calponin H1, smooth muscle	CNNh1	557012	1.27	2.10
Apoptosis				
Apoptotic protease-activating factor 1	APAF-1	657503	1.33	2.20
Seven in absentia 1B	SIAH-1B	618379	1.30	2.04
Cellular processes				
Microtubule-associated protein tau	TAU	552102	1.36	2.23
Integrin α-6	Iα6	584662	1.63	2.05
Cytochrome P450, 2d10			1.46	2.34
Others				
PRL receptor	PRL-R	520835	0.72	3.74
EST, highly similar to ENV polyprotein precursor		539102	1.79	2.20
δ-Aminolevulinate dehydratase	DAH	518879	1.53	2.13

insulin increased levels of α-5 integrin gene expression in mouse fibroblast (NIH-3T3) cells. Interestingly, genes that inhibit cell growth as well as genes that enhance cell growth were simultaneously activated in response to IGF-1 stimulation. For example, IGF-1 treatment increased the expression of the early growth response-1 transcription factor, which is associated with proliferation, but it also increased the expression of nerve growth factor-induced clone A-binding protein 2, which is a corepressor protein that can repress the transcription of genes targeted by early growth response-1 (43). Similarly, although IGF-1 induces proliferation, IGF-1 also increased expression of the Tel oncogene, which has been shown to retard cell proliferation of many cell types, particularly fibroblasts (44). These findings suggest that there are many antiproliferative processes that are regulated by IGF-1 and that cell growth is a tightly controlled process.

In our cDNA microarray analysis, IGF-1 also increased the expression of several genes involved in specific cellular pro-

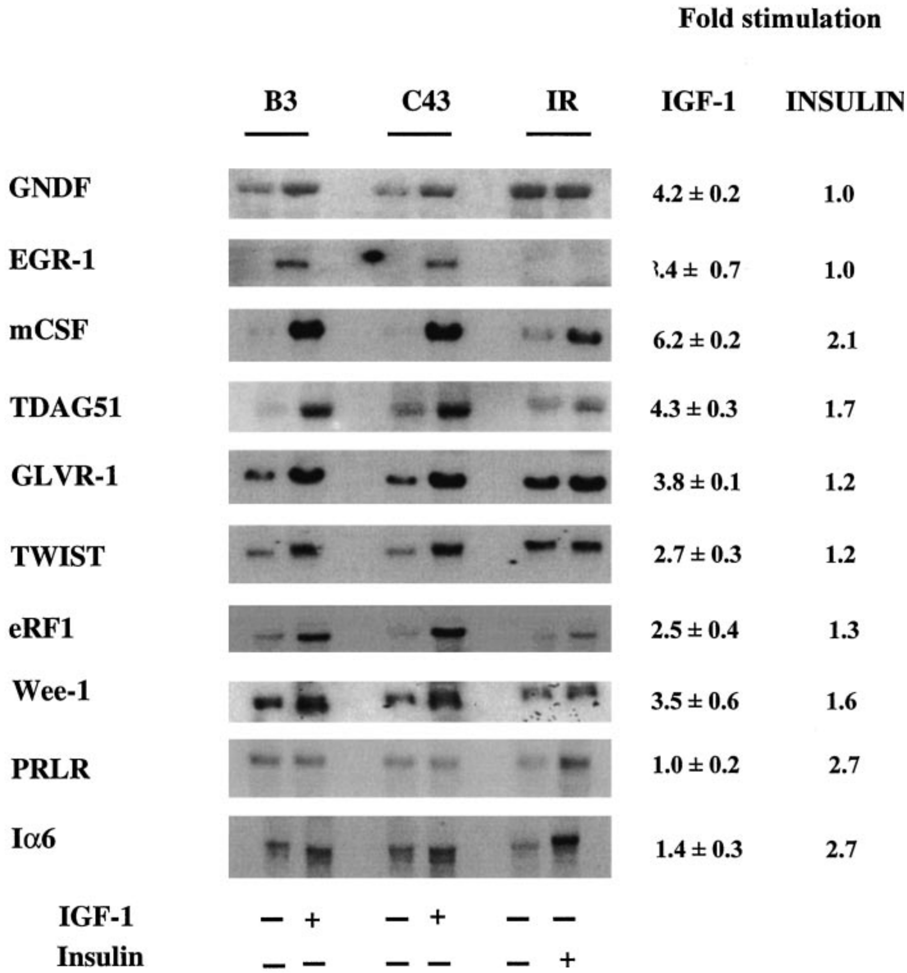

FIG. 2. Northern blots of genes differentially induced by IGF-1 or insulin. Northern blots were used to confirm the changes in gene expression induced by IGF-1 and insulin on cDNA microarrays. NIH-3T3 cells expressing the human IGF-1R [NWTb3 (B3) and NWTc43 (C43) cells] or NIH-3T3 cells expressing the human IR (IR) were starved for 16 h and then incubated in the presence or absence of 50 nM IGF-1 or insulin for 90 min, respectively. RNA was then extracted from cells, and samples containing 20 μg of total RNA were analyzed by Northern blotting as described in *Materials and Methods*. Probes were generated from DNA fragments from the indicated genes as described. In each case, the membrane was stripped and reprobed with the 18S RNA to confirm equal loading and to quantify signal intensity. All Northern blot procedures were repeated twice. Data are shown as mean (fold increase from control) ± SEM for IGF-1 (n = 4, the values for the two clones from two separate experiments were combined) and insulin (n = 2, *i.e.* two separate experiments) as indicated. The abbreviations used for the various genes are defined in Tables 1 and 2.

TABLE 3. Genes up-regulated by both IGF-1 and insulin

	Symbol	Clone number	IGF-1	Insulin
Parvalbumin	PAR	493697	3.30	2.31
Myosin heavy chain, skeletal muscle	MyoHC	440791	3.15	2.30
Zinc finger protein 90	ZFP-90	463944	2.82	2.32
TNF receptor 1a	TNF-R1a	552363	2.80	2.35
EST		584397	2.74	2.20
EST		585341	2.41	2.20
Splicing factor, arginine/serine-rich 10	SRp10	540863	2.40	2.30
Colony-stimulating factor, granulocyte receptor	CSF,GR	586299	2.40	2.25
Kidney androgen-regulated protein	KARp	581191	2.40	2.20
Tubulin, β-2	Tub2	585507	2.35	2.22
Adenine nucleotide translocator-2, fibroblast	ANT-2	585992	2.30	2.24
Von Hippel-Lindau syndrome homolog	VHL	573081	2.22	2.24
Transgelin	TRANS	603669	2.20	2.24

cesses, including cell division, chromosome partitioning, and protein translation, which are all critical for cell growth (Table 1). It has been well established that IGF-1 regulates the determination of several cell lineages. Indeed, we found that IGF-1 induced the expression of several transcription factors involved in cell differentiation, including Forkhead homolog 14 (45), SRY box-containing gene 2 (46), and Twist (47). Insulin treatment increased the expression of α-B crystallin (48) and calponin H1 (49), which are involved in the organization and protection of myofibrillar structure. Although insulin-responsive genes are not generally classified as mitogenic, we cannot exclude a role for insulin in cell growth. However, our data suggest that IGF-1 and insulin could exert distinct regulatory effects on cellular proliferation, differentiation, and morphogenesis. It is well known that the IGF-1R plays an antiapoptotic role in fibroblasts (50). However, in the mouse blastocyst, high concentrations of IGF-1 can actually trigger apoptosis by down-regulating the IGF-1R (51). In this study, we found that IGF-1 increased expression of the antiapoptotic Twist gene and concomitantly increased expression of two proapoptotic genes, T cell death-associated gene 51 and Fas-binding protein genes. This further suggests that

TABLE 4. Genes down-regulated by both IGF-1 and insulin

	Symbol	Clone number	IGF-1	Insulin
FK506-binding protein 1a	FK504bp	604923	0.14	0.19
Thymoma viral protooncogene	TVP	616283	0.37	0.43
EST		557037	0.42	0.39

during stimulation with IGF-1, the balance between cell death and cell survival is tightly regulated. We have used Northern blots to verify the regulation of many, but not all, of the 39 IGF-1- and insulin-regulated genes identified on microarrays. In some cases, the ratio of signal intensities on microarrays was slightly greater than the threshold level of 2.0. The regulation of α-6 integrin (up-regulated 2.05-fold by insulin) and Wee 1-like protein kinase (up-regulated 2.75-fold by IGF-1) was confirmed by Northern blot analysis, suggesting that these relatively modest changes reflect authentic changes in gene expression. However, other genes that were modestly regulated by microarray analysis (~2-fold) have not yet been confirmed by Northern blot, including MAK16, DPA, EDR, and an EST highly similar to ENV. Thus, the data for this group of genes must be interpreted with caution. Another caveat to be considered is that the various cell lines express different levels of IR and IGF-1R and these differences could affect responses, although the concentration of ligand was physiological and not likely to bind the other receptor. IGF binding proteins are expressed at relatively low levels compared with IGF-1Rs in the NWTb3 cells and are also unlikely to affect the responses to IGF-1.

In summary, many genes were differentially regulated by equivalent doses of IGF-1 and insulin (*i.e.* NWTb3 cells or IR cells were exposed for 90 min to either 50 nM IGF-1 or 50 nM insulin). Thus, the specificity of insulin and IGF-1 signaling may be mediated, at least in part, by the induction of different patterns of gene expression by activation of the IR and IGF-1R. Interestingly, some studies, albeit in other cell types and under different conditions, have shown that IGF-1 and insulin can act on the same genes but with different outcomes. For example, in murine keratinocytes, insulin induces the expression of classic markers of differentiation, whereas IGF-1 stimulation inhibits the expression of these same markers (52). Also, in the developing eye lens of the chicken, the level of δ-crystallin induced by IGF-1 is greater and occurs more quickly than that induced by insulin (53). To explain some of the differential effects of insulin and IGF-1, some investigators have searched for substrates that may be specific for either receptor. For example, Najjar *et al.* (54) showed that the IR but not the IGF-1R interacts with and phosphorylates pp120 (also known as C-CAM or Caecam-1), a plasma membrane glycoprotein that plays a role in endocytosis of the insulin/IR complex. Laviola *et al.* (55) showed that in mouse fibroblasts, the adapter protein Grb10 preferentially associates with the IR compared with the IGF-1R and therefore might contribute to the specificity of the biological effects of the two hormones. Some reports also speculate that the IR and IGF-1R could activate different signaling pathways to trigger either the same or different responses. Other theories have also been proposed to explain the difference between IR and IGF-1R signaling. Some have suggested that the different patterns of tissue distribution of these receptors influence the physiological responses that they exert (56), and others have argued for a functional role of hybrid receptors (57). Finally, some investigators have favored the explanation that the different receptors generate qualitatively different signals, for example, in the subcellular distribution (58) or duration (59) of the stimulus. In our study, the differential effect maybe attributable to the fact that the basal levels in the various cell lines were different; glial cell line-derived neurotrophic factor and Gibbon ape leukemia virus receptor-1 in the IR cells and α-6 integrin in the NWTB3 cells were quite increased. Consequently, the stimulation by the ligands may be blunted. Finally, it is important to note that in addition to the distinct effects of IGF-1 and insulin, we also found that a number of genes are similarly increased or decreased by these two hormones (Tables 3 and 4).

In conclusion, we have used cDNA microarray technology to compare the gene expression profiles induced by insulin and IGF-1. We identified 39 target genes, most of which have not been previously described. Thirty genes were up-regulated specifically by IGF-1 and not by insulin, whereas only 9 genes were up-regulated by insulin and not by IGF-1. Half of the genes specifically regulated by IGF-1 are associated with mitogenesis and differentiation. Thus, under equivalent conditions in mouse fibroblast NIH-3T3 cells, IGF-1 appears to induce more genes associated with mitogenesis than does insulin. Furthermore, our findings increase the known set of genes regulated by IGF-1 and insulin. Moreover, in a separate study, we showed that Twist, which was identified by microarray analysis as a specific IGF-1-responsive gene, is involved in the antiapoptotic effects of the IGF-1R in mouse fibroblasts (60). Thus, characterization of the gene expression profiles induced by insulin and IGF-1 has allowed us to identify a novel component involved in one of the critical functions of the IGF-1R. In future studies, it will be of interest to examine the specific roles played by the other genes identified in this study in the overall biological functions of the IGF-1R and IR.

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Address all correspondence and requests for reprints to: Derek LeRoith, M.D., Ph.D., Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Room 8D12, Building 10, NIH, Bethesda, Maryland 20892-1758. E-mail: derek@helix.nih.gov.

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